Journal of Food Protection, Vol. 68, No. 2, 2005, Pages 282–291

Studies To Select Appropriate Nonpathogenic Surrogate *Escherichia coli* Strains for Potential Use in Place of *Escherichia coli* O157:H7 and *Salmonella* in Pilot Plant Studies†

DENISE R. EBLEN,‡§ BASSAM A. ANNOUS,‡* AND GERALD M. SAPERS

Food Safety Intervention Technologies, Eastern Regional Research Center, U.S. Department of Agriculture, Agricultural Research Service, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 04-80: Received 27 February 2004/Accepted 6 September 2004

ABSTRACT

The response of a potential nonpathogenic surrogate organism to a particular treatment should closely mimic the response of the target pathogenic organism. In this study, growth characteristics (generation time, lag phase duration, and maximum population), pH at stationary phase, and survival characteristics (level of attachment and survival on apple surfaces, resistance to hydrogen peroxide decontamination treatments, and thermal resistance at 60°C) of 15 nonpathogenic generic *Escherichia coli* strains and one nonpathogenic *E. coli* O157:H43 strain were compared with those of two *E. coli* O157:H7 strains and two *Salmonella* strains. Few differences in growth characteristics or pH at stationary phase were evident between nonpathogenic and pathogenic strains tested. However, considerably more separation among strains was seen following investigation of survival characteristics. *E. coli* ECRC 97.0152, which does not contain genes encoding for known virulence factors associated with *E. coli* O157:H7, appears to be a good surrogate candidate, with growth and survival characteristics similar to those of *E. coli* O157:H7 strains. The less heat-resistant surrogate strains *E. coli* NRRL B-766 and NRRL B-3054 and *E. coli* ATCC 11775, ATCC 25253, and ATCC 25922 may be used when attempting to model the heat resistance of *Salmonella* Montevideo G4639 and *Salmonella* Poona RM 2350, respectively. These surrogate strains may be useful for evaluating the efficacy of intervention steps in reducing populations of selected strains of *E. coli* O157:H7 and *Salmonella* in processing environments where these pathogens cannot be introduced.

Researchers in the field of applied microbial food safety have many factors to consider when designing validation and challenge studies. Often, the goal of such research is to characterize the behavior of a particular pathogen in a target food of food processing step with a view to developing a way of eliminating or controlling pathogen proliferation. Although the use of the intended target pathogen would be ideal, researcher safety concerns must also be considered. In many instances, researchers find it necessary to use a nonpathogenic surrogate organism in place of the target pathogenic organism. Such studies are inherently limited because surrogate organisms may not behave in a manner identical to that of their pathogenic counterparts. Nonpathogenic surrogate organisms do not have the virulence genes of wild-type outbreak strains, i.e., the type of strains whose behavior and responses the research is attempting to reproduce. Pathogenic strains also may have unique characteristics that contribute to their virulence. For example, *Escherichia coli* O157:H7 is highly tolerant of acidic conditions *(8)*, a factor that has been cited as contributing to the virulence of this pathogen by permitting survival in the low pH of the stomach *(12)*. The U.S. Food and Drug Administration *(11)* defined a surrogate microbe as ''a nonpathogenic species and strain responding to a particular treatment in a manner equivalent to a pathogenic species and strain. The surrogate allows biological verification of the treatment without introducing pathogens into a food processing area.'' Therefore, potential nonpathogenic surrogate organisms should be characterized prior to use in validation and challenge studies and should be negative for virulence factors of target pathogens relevant to the food system of interest.

Our laboratory is engaged in pilot plant studies to characterize the efficacy of various washing and sanitizing treatments in reducing populations of pathogenic microflora, specifically *E. coli* O157:H7 and *Salmonella*, on fresh fruit and vegetables. Because these organisms cannot be introduced to the pilot plant in its present configuration because of concerns for the safety of equipment operators or other personnel in the pilot plant area, nonpathogenic organisms are needed. The aim of the present study was to match the growth, survival, and thermal resistance characteristics of 15 nonpathogenic generic *E. coli* strains and one nonpathogenic O157:H43 *E. coli* strain with characteristics of two strains each of *E. coli* O157:H7 and *Salmonella*. All four pathogenic strains were previously associated with foodborne outbreaks in produce or apple cider. Growth response and thermal inactivation of bacteria were previously shown

* Author for correspondence. Tel: 215-233-6797; Fax: 215-233-6406; E-mail: bannous@arserrc.gov.

† Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

‡ Annous and Eblen contributed equally to this work and are considered co-senior authors.

§ Present address: USDA/FSIS/OPHS, 1400 Independence Avenue S.W., Washington, DC 20250, USA.

TABLE 1. *Bacterial strains*

Strain[a]	Source
E. coli O157:H43[b]	Dr. D. Debroy, Penn State University, University Park, Pa.
E. coli ECRC 96.0509[c]	Dr. D. Debroy, Penn State University, University Park, Pa.
E. coli ECRC 97.0147[c]	Dr. D. Debroy, Penn State University, University Park, Pa.
E. coli ECRC 97.0152[c]	Dr. D. Debroy, Penn State University, University Park, Pa.
E. coli ECRC 97.0190[c]	Dr. D. Debroy, Penn State University, University Park, Pa.
E. coli ECRC 99.0512[c]	Dr. D. Debroy, Penn State University, University Park, Pa.
E. coli ECRC 99.1232[c]	Dr. D. Debroy, Penn State University, University Park, Pa.
E. coli ATCC 11775[d]	Dr. P. Fratamico, USDA-ARS-ERRC, Wyndmoor, Pa.
E. coli ATCC 25253	Dr. R. Merker, FDA-CFSAN, Washington, D.C.
E. coli ATCC 35695	Dr. R. Merker, FDA-CFSAN, Washington, D.C.
E. coli ATCC 25922	Dr. W. Fett, USDA-ARS-ERRC, Wyndmoor, Pa.
E. coli NRRL B-766	Dr. L. K. Nakamura, USDA, National Center for Agricultural Utilization Research (NCAUR), Peoria, Ill.
E. coli NRRL B-2783	Dr. L. K. Nakamura, USDA, NCAUR, Peoria, Ill.
E. coli NRRL B-3054	Dr. L. K. Nakamura, USDA, NCAUR, Peoria, Ill.
E. coli NRRL B-3704	Dr. L. K. Nakamura, USDA, NCAUR, Peoria, Ill.
E. coli NRRL B-14573	Dr. P. Fratamico, cider outbreak strain
E. coli O57:H7 SEA 13B88	Dr. M. Lytle, Oklahoma State Department of Health, Oklahoma City; cider outbreak strain
E. coli O157:H7 OK	
Salmonella Poona RM 2350	Dr. W. Fert, cantaloupe outbreak strain
Salmonella Montevideo G4639	Dr. L. Beuchat, University of Georgia, Griffin; tomato outbreak strain

[a] ECRC, *E. coli* Reference Center, University Park, Pa.; ATCC, American Type Culture Collection; NRRL, Northern Regional Research Laboratory, Peoria, Ill.
[b] Nonpathogenic strain.
[c] All O55 strains.
[d] Contains green fluorescent protein on pGFPuv plasmid.

to be dependent on environmental parameters such as the growth medium *(2, 3, 5).* In this study, the growth profiles and thermal resistance of all strains were examined following incubation in five typical growth media. Ongoing studies at our laboratory concern novel methods of decontaminating fruit with H_2O_2 and other antimicrobial agents; therefore, the level of attachment of the test strains to apple surfaces and their subsequent reduction by washing with a H_2O_2 solution were also investigated. Our goal was to identify a nonpathogenic surrogate that would not pose a health hazard and whose behavior closely resembled that of the pathogens represented under the parameters examined.

MATERIALS AND METHODS

Strains. Fifteen non-O157:H7 *E. coli* strains, one nonpathogenic *E. coli* O157:H43 strain, two *E. coli* 0157:H7 outbreak strains, and two *Salmonella* outbreak strains were used (Table 1). *E. coli* O157:H43, a nonpathogenic mutant of O157:H7, was included for comparison purposes only and was not considered as a possible surrogate. All strains were stored in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) containing 20% (vol/vol) glycerol at −80°C. Working stocks were kept on tryptic soy agar (TSA; Difco, Becton Dickinson) slants containing 0.6% (wt/vol) yeast extract (Difco, Becton Dickinson) at 4°C for 2 weeks.

All *E. coli* strains were confirmed by streaking on eosin methylene blue agar (Difco, Becton Dickinson) on which *E. coli* produces a green sheen with reflected light. *E. coli* O157:H7 and O157:H43 colonies from each study were further identified using the RIM *E. coli* O157:H7 latex agglutination test (Remel, Lenexa, Kans.), which identifies the O157 and H7 antigens. *Salmonella* strains were confirmed using a commercial latex agglutination test (Oxoid, Basingstoke, UK), which identifies the flagellar antigens.

All potential surrogate strains included in the study were submitted to the Gastroenteric Disease Center (Wiley Lab, Pennsylvania State University, University Park, Pa.) and tested for the presence of genes encoding for known virulence factors associated with *E. coli* O157:H7: (i) STa, heat stable toxin; (ii) STb, heat stable toxin; (iii) LT, heat labile toxin; (iv) SLT-I, Shiga-like toxin I; (iv) SLT-II, Shiga-like toxin II; (v) K99, fimbrial adhesion factor; (vi) *eae*, attaching and effacing gene; (vii) F1845, fimbrial gene; and (viii) CS31A, attachment factor.

Preparation of growth media. Five different growth media were prepared according to the manufacturers' instructions: (i) TSB (standard formulation contains 0.25% glucose); (ii) TSB+G (TSB supplemented with glucose [Sigma; St. Louis, Mo.] to a final concentration of 1% [wt/vol]); (iii) TSB−G (TSB with no glucose; Difco, Becton Dickinson); (iv) TSB−GHCl (TSB−G adjusted to pH 5.0 with 0.1 M HCl); and (v) brain heart infusion broth (BHI; Difco, Becton Dickinson). Initial pH of uninoculated TSB, TSB−G, TSB+G, and BHI was 7.4 to 7.6. BHI and TSB were chosen as typical growth media. TSB+G was included because it has been reported that the acid tolerance of *E. coli* O157:H7 is enhanced during growth in the presence of glucose in this manner because of adaptation to gradual production of acid by the metabolizing cells *(6).* However, such growth conditions can result in a significant amount of injury to growing cells *(6, 14),* which may impair their subsequent responses to stress. Therefore, the magnitude of the effect of glucose and acidity was investigated by growing the cells in TSB that contained no glucose (TSB−G) and by growing the cells in TSB−G in which HCl (an inorganic acid) was present as the acidulent (TSB−GHCl). The pH of each

broth was measured in triplicate after preparation and after cell growth of each strain reached the stationary phase.

Growth characteristics. Individual strains were grown in 10 ml of TSB at 37°C for 16 h with shaking at 75 rpm. The active culture was then diluted 1:100 with sterile 0.1% (wt/vol) peptone water (PW; Difco, Becton Dickinson), and 1 μl of this dilution was transferred to triplicate 30-ml volumes of each of the five growth media (TSB, TSB+G, TSB−G, TSB−GHCl, and BHI) in 250-ml Erlenmeyer flasks. The flasks were incubated at 37°C with shaking at 75 rpm. Populations were enumerated by aseptically removing a 0.1-ml aliquot of the culture at 0, 1, 2, 4, 6, 8, 10, and 24 h, diluting in 0.1% (wt/vol) PW as necessary, and surface plating on TSA. TSA plates were incubated overnight at 37°C prior to counting. Growth curves were generated by fitting the Gompertz function to the data *(21).* The Gompertz function parameters were then used to calculate the generation time, lag phase duration, and maximum population density (log CFU per milliliter) for each strain in each growth medium.

Attachment and survival on apple surfaces and resistance to H_2O_2 wash treatments. Individual strains were grown in 10 ml of TSB at 37°C for 8 h and used to inoculate 1 liter of TSB at 0.01% (vol/vol) concentration, which was then incubated at 37°C for 16 h with shaking at 75 rpm. Cells were recovered by centrifugation at 11,170 × *g* for 10 min at 4°C, washed once with 250 ml of PW, and suspended in 2 liters of sterile distilled water. This cell suspension was used to inoculate apples. Initial cell populations were determined by serially diluting the culture in PW and surface plating duplicate 0.1-ml aliquots on TSA.

Unwaxed Golden Delicious apples (32 per experiment) obtained from a single Washington State grower were removed from storage at 4°C and inoculated in less than 1 h with the strain under investigation by submerging apples (six at once) in 2 liters of inoculum at 20°C for 5 min. The apples were placed on their sides to allow for drainage from the stem and calyx areas and left to air dry for 2 h on absorbent paper on the bench top. At this point, half of the apples ($n = 16$) were stored at 4°C for 24 h. The remaining apples were divided into two sets of eight. The first set was divided into duplicate composite sets of four apples. Each apple was weighed and cut into quarters using a sterile knife and cutting board. Apple pieces of each composite set were placed in a 4-liter stainless steel blender (Waring, Torrington, Conn.) with a volume of PW equal to the apple weight and blended on low speed for 1 min. The resulting blend was filtered through a filter stomacher bag (Spiral Biotech, Bethesda, Md.), and duplicate 10-ml volumes were transferred to sterile tubes. This procedure was repeated with the second composite set of fruit. The remaining eight apples were separated into two sets of four apples, and each set of apples was washed with 5% (vol/vol) H_2O_2 solution at room temperature (prepared from a 30% [vol/vol] H_2O_2 solution; Fisher Scientific, Pittsburgh, Pa.) with shaking for 1 min, briefly drained, and immediately blended. The entire procedure was repeated for apples stored for 24 h. Filtrates were diluted in PW as necessary and surface plated on the appropriate enumeration agar medium. Uninjured cells of nonpathogenic *E. coli*, *E. coli* O157:H7, and *Salmonella* were enumerated on selective MacConkey agar (Difco, Becton Dickinson), sorbitol MacConkey agar (Difco, Becton Dickinson), and xylose lysine Tergitol 4 agar (Difco, Becton Dickinson), respectively. Recovery medium (TSA) was used for enumeration of injured *E. coli* and *Salmonella* cells. After inoculation, TSA plates were incubated at 37°C for 2 h to allow recovery of injured cells and were then overlaid with the appropriate selective medium *(10).* All plates were incubated overnight at 37°C, and resultant colonies were manually counted.

Thermal inactivation. The *D*-value (time to reduce bacterial populations by 1 log or 90%) of each organism under investigation was determined at 60°C ($D_{60°C}$). Individual strains were grown in 10 ml of TSB at 37°C for 8 h with shaking at 75 rpm and were used to inoculate 30 ml of each of the five growth media (TSB, TSB+G, TSB−G, TSB−GHCl, and BHI) at 0.01% (vol/vol) concentration. Cells were grown for 16 h with shaking at 75 rpm at 37°C, recovered by centrifugation, washed once with PW, and suspended in 30 ml of sterile PW. Samples with similar cell densities were heat treated at 60°C by the method of Cole and Jones *(7),* using a Techne submerged-coil heating apparatus model tempette TE-8D (Protocol Instruments Ltd., West Byfleet, UK). Each thermal inactivation procedure was performed in triplicate. Heat-treated samples were serially diluted in PW, and duplicate 0.1-ml aliquots were surface plated on TSA. Plates were incubated overnight at 37°C prior to counting. The *D*-value was calculated by plotting log number of survivors against time and obtaining the reciprocal of the slope of the line using Excel spreadsheet software (Microsoft Corporation, Redmond, Wash.).

Statistical analyses. Analyses of variance with individual contrasts and Bonferroni *t* tests were performed to determine significant differences between population means in response to strain and medium and to determine medium-strain interactions. Comparisons of final pH values, growth parameters, attachment and resistance to H_2O_2 wash, and thermal inactivation for each strain in each medium were made using the Bonferroni least significant difference separation technique. All statistical analyses and calculations of means and standard deviations were performed using SAS/STAT software (SAS Institute Inc., Cary, N.C.).

RESULTS

Nonpathogenicity confirmatory tests. All nonpathogenic *E. coli* strains included in the study were tested for the presence of virulence genes associated with *E. coli* O157:H7. All nonpathogenic *E. coli* strains were negative for all virulence genes tested, with the exception of strain NRRL B-2783, which was positive for the O157 gene. Consequently, this strain and the O157:H43 strain were not considered as potential surrogates in the final analysis.

Changes in broth pH following microbial growth. The pH values of uninoculated broths incubated as controls decreased by 0.04 to 0.11 units following incubation overnight at 37°C. Every strain, unless otherwise noted, after being grown in broth exhibited the following relationship of final pH: TSB−G > BHI = TSB > TSB−GHCl > TSB+G ($P < 0.05$) (Table 2). An exception to this pattern was seen with *E. coli* NRRL B-14573, where a lower pH was obtained after growth in TSB−GHCl compared with growth in TSB+G, and no difference was observed in final pH between TSB and TSB+G (data not shown). No significant difference in final pH ($P < 0.05$) was noted for TSB−GHCl and BHI after growth of *E. coli* ECRC 96.0509 (data not shown). For *Salmonella* Poona, there was no difference in the final pH ($P < 0.05$) after growth in BHI, TSB, and TSB−GHCl, whereas for *Salmonella* Montevideo growth in BHI resulted in a significantly higher final pH ($P < 0.05$) than growth in TSB and TSB−GHCl, whose final pH did not differ significantly ($P < 0.05$) (Table 2).

Table 2 lists the potential surrogate strains with final

TABLE 2. *Surrogate strains with significantly different ($P < 0.05$) final pH values compared with pathogenic strains*[a]

Pathogenic strain	Growth medium: BHI		TSB		TSB+G		TSB−G		TSB−GHCl	
	Surrogate strain	pH[b]	Surrogate strain	pH	Surrogate strain	pH	Surrogate strain	pH	Surrogate strain	pH
E. coli O157:H7 SEA 13B88	96.0509[c]	6.00 ± 0.03	NS[d]	5.97 ± 0.05	B-14573[e]	4.77 ± 0.01	B-14573[e]	7.10 ± 0.03	NS	5.45 ± 0.02
E. coli O157:H7 OK	96.0509[c]	6.00 ± 0.04	NS	5.96 ± 0.03	B-14573[e]	4.74 ± 0.02	NS	7.09 ± 0.03	NS	5.41 ± 0.03
Salmonella Montevideo G4639	SL[f]	6.56 ± 0.86	B-14573[c] 96.0509[c]	6.08 ± 0.06	B-14573[e]	4.70 ± 0.06	25253[c]	7.35 ± 0.04	SL	5.93 ± 0.06
Salmonella Poona RM 2350	96.0509[c]	5.98 ± 0.04	B-14573[c]	6.07 ± 0.06	B-14573[e]	4.71 ± 0.02	NS	7.35 ± 0.02	SL	5.86 ± 0.06

[a] All *E. coli* strains, identified by strain numbers (see Table 1).
[b] Mean ± standard deviation.
[c] Significantly lower final pH ($P < 0.05$) than that of pathogenic strain.
[d] NS, no significant difference ($P < 0.05$) in final pH values between surrogate strains tested (Table 1) and the pathogenic strain.
[e] Significantly higher final pH ($P < 0.05$) than that of pathogenic strain.
[f] SL, all surrogate strains tested (Table 1) had significantly lower final pH ($P < 0.05$) than that of the pathogenic strain.

pH values significantly different ($P < 0.05$) from those of pathogenic strains following growth in each medium. Little separation was evident, with some exceptions. Both *Salmonella* strains had significantly higher final pH values ($P < 0.05$) than did *E. coli* O157:H7 strains in TSB−GHCl. All surrogate strains tested had a significantly lower final pH ($P < 0.05$) than that of *Salmonella* Montevideo G4639 after growth in BHI or TSB−GHCl. All surrogate strains tested had a significantly lower final pH ($P < 0.05$) than that of *Salmonella* Poona RM 2350 after growth in TSB−GHCl. The final pH of all surrogate strains grown in TSB−G was not significantly different ($P < 0.05$) from those of *Salmonella* Poona RM 2350. *E. coli* NRRL B-14573 grown in TSB or TSB+G had significantly lower and higher ($P < 0.05$) final pH, respectively, compared with both *Salmonella* strains. *E. coli* ECRC 96.0509 had a significantly lower final pH ($P < 0.05$) than did *Salmonella* Montevideo G4639 after growth in TSB and *Salmonella* Poona RM 2350 after growth in BHI. *E. coli* ATCC 25253 had a significantly lower final pH ($P < 0.05$) than did *Salmonella* Montevideo G4639 after growth in TSB−G. The final pH of both *E. coli* O157:H7 strains was not significantly different from that of the other microorganisms tested, with the following exceptions: *E. coli* ECRC 96.0509 had a significantly lower final pH ($P < 0.05$) in BHI and *E. coli* NRRL B-14573 had a significantly higher final pH ($P < 0.05$) in TSB+G. The latter strain also had a significantly higher final pH ($P < 0.05$) after growth in TSB−G than did *E. coli* O157:H7 SEA 13B88.

Growth characteristics. The analysis of three growth parameters (generation time, lag phase duration, and maximum population) was performed to determine the effects of strains and growth media. Generation times for the potential surrogate strains that were significantly different ($P < 0.05$) from pathogenic strains are shown in Table 3. Overall, there was no significant difference in generation times for all strains grown in BHI, TSB, TSB+G, and TSB−G, with the exception of *E. coli* NRRL B-14573 grown in BHI or TSB−G, which had a significant longer generation time ($P < 0.05$). Significantly longer generation times ($P < 0.05$) were seen when strains were grown in TSB−GHCl than when they were grown in the other broths tested, with the exception of *E. coli* ECRC 99.1232. Overall, *E. coli* ECRC 99.0512 grown in TSB−GHCl had the shortest generation time (0.09 h) among strains and media tested ($P < 0.05$). There was no significant difference in lag phase duration between the strains in any of the media (range, 1.56 to 2.55 h; data not shown). Significantly longer lag phases ($P < 0.05$) were seen when strains were grown in TSB−GHCl than when they were grown in the other broths tested (data not shown). Overall, the highest and lowest maximum population densities of all bacterial strains tested were obtained following growth in BHI and TSB and in TSB−GHCl, respectively (data not shown). Exceptions were the *E. coli* NRRL B-2783, NRRL B-3704, and NRRL B-766 strains, where the maximum population densities were obtained following growth in TSB−GHCl (data not shown). These population densities were not significantly

TABLE 3. *Surrogate strains with significantly different (P < 0.05) generation times compared with pathogenic strains grown in different growth media*

Strain	Generation time (h)[a]				
	BHI	TSB	TSB+G	TSB−G	TSB−GHCl
Pathogenic					
E. coli O157:H7 SEA 13B88	0.24 ± 0.02 A	0.25 ± 0.04 A	0.25 ± 0.03 A	0.24 ± 0.02 A	0.47 ± 0.09 A
E. coli O157:H7 OK	0.21 ± 0.04 A	0.24 ± 0.02 A	0.24 ± 0.03 A	0.21 ± 0.04 A	0.48 ± 0.02 A
Salmonella Montevideo G4639	0.28 ± 0.04 A	0.27 ± 0.02 A	0.17 ± 0.03 A	0.28 ± 0.04 A	0.49 ± 0.05 A
Salmonella Poona RM 2350	0.23 ± 0.03 A	0.21 ± 0.03 A	0.21 ± 0.02 A	0.23 ± 0.03 A	0.41 ± 0.08 A
Surrogate[b]					
E. coli ATCC 25253	NS[c]	NS	NS	NS	0.62 ± 0.02 B
E. coli NRRL B-3704	NS	NS	NS	NS	0.57 ± 0.05 B
E. coli NRRL B-14573	0.43 ± 0.03 B	NS	NS	0.43 ± 0.03 B	0.64 ± 0.07 B
E. coli ECRC 99.0512	NS	NS	NS	NS	0.09 ± 0.11 C
E. coli ECRC 99.1232	NS	NS	NS	NS	0.27 ± 0.04 D

[a] Each value represents the mean ± standard deviation of three trials. Means within each column with the same letter are not significantly different ($P < 0.05$).

[b] Surrogate strains not listed (see Table 1) showed no significant difference ($P > 0.05$) in generation time as compared with the pathogenic strains.

[c] NS, data not shown because values were not significantly different ($P > 0.05$) from generation times for the pathogenic strains.

different ($P < 0.05$) from each other nor was there any difference among the maximum population densities achieved in the other growth broths (data not shown). Overall, the growth characteristics of the pathogens were not significantly different from each other or, with few exceptions, from those of the potential surrogates.

Attachment and survival on apple surfaces and resistance to H_2O_2 wash treatments. Cell counts from treated apples were determined on recovery and selective media. Cell counts on recovery media were generally higher than counts on selective media, although significantly so only for potential surrogate *E. coli* strains ATCC 11775, ATCC 35695, NRRL B-14573, NRRL B-3054, NRRL B-3704, NRRL B-766, and ECRC 99.1232 and *Salmonella* Poona RM 2350 and *Salmonella* Montevideo G4639 ($P < 0.05$).

Table 4 lists the potential surrogate strains that had equivalent or greater attachment and survival ($P < 0.05$) on apple surfaces than did each pathogenic strain, with and without the H_2O_2 wash treatment, on day 0 and day 1. There were significant interactions between the days on which survivors were enumerated and whether or not the apples were treated with the 5% H_2O_2 wash ($P < 0.05$). Overall, samples from treated and untreated apples showed significantly higher counts on day 0 than on day 1 ($P < 0.05$). Reductions in cell numbers following 5% H_2O_2 wash treatment were only significant ($P < 0.05$) for apples sampled on day 0.

Thermal inactivation. Survivor curves demonstrated a linear decrease in cell numbers with time during heating at 60°C (curves not shown). Table 5 lists potential surrogate strains that had equivalent or greater $D_{60°C}$-values ($P < 0.05$) than did pathogenic strains. There was a significant interaction between the strains tested and the growth medium used ($P < 0.05$). The $D_{60°C}$-value of *E. coli* O157:H7 SEA 13B88 after growth in TSB+G was significantly higher ($P < 0.05$) than values obtained following growth in other growth media tested. Both *E. coli* O157:H7 strains were generally more thermal resistant than *Salmonella* strains ($P < 0.05$). However, there were two exceptions to this trend; following growth in TSB−G and in TSB−GHCl, *Salmonella* Montevideo G4639 did not significantly differ from *E. coli* O157:H7 SEA 13B88 ($P < 0.05$).

DISCUSSION

This study was a first attempt to match the growth characteristics, thermal resistance, attachment to produce, and resistance to a sanitizer wash treatment of nonpathogenic surrogate organisms to those of pathogenic counterparts. The lag phase duration observed for *E. coli* O157:H7 SEA 13B88 was considerably (28.6-fold) shorter than that reported by Whiting and Golden *(22)* for the same strain. These researchers grew the culture at 15°C in BHI containing 1.5% NaCl (pH 5.3), which could explain the longer lag phase duration. The final pH values for *E. coli* O157:H7 SEA 13B88 after growth in TSB+G and TSB−G (Table 2) were in agreement with those previously reported by Buchanan and Edelson *(6)*. Investigation of growth characteristics and final pH at stationary phase gave an indication of the metabolic behavior of each potential surrogate in each broth compared with the pathogenic strains. Very few differences were seen, which was not surprising given that all the strains tested are members of the *Enterobactericeae* and as such have similar responses to such test conditions, which are not inherently stressful. Therefore, any of the nonpathogenic *E. coli* strains tested had the potential to act as a surrogate organism for pathogenic strains tested with respect to growth characteristics and final pH value under these conditions. Considerably more separation among strains was seen following investigation of attach-

TABLE 4. *Surrogate strains that demonstrate attachment and survival equivalent to or greater than that of the pathogenic strains (*$P < 0.05$*) with and without the 5%* H_2O_2 *washing treatment on days 0 and 1*[a]

Pathogenic strain	Day 0		Day 1	
	Control	Washed	Control	Washed
E. coli O157:H7 SEA 13B88	(4.67 ± 0.66) A B-14573, 25253, 25922, B-2783,[b] 35695, B-3704, B-766, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512, 99.1232, O157:H43[b]	(3.15 ± 0.29) B 11775, B-14573, 25253, 25922, B-2783, B-3054, 35695, B-3704, B-766, 96.0509, 97.0147, 97.0152,[c] 97.0190, 99.0512, 99.1232, O157:H43	(4.28 ± 0.41) A B-14573, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512, 99.1232	(2.89 ± 0.62) B 11775, B-14573,[c] 25253, 25922, B-2783, B-3054, 35695, B-3704, B-766, 96.0509, 97.0147, 97.0152,[c] 97.0190, 99.0512, 99.1232
E. coli O157:H7 OK	(4.70 ± 0.48) A B-14573, 25253, B-2783, B-3704, B-766, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512, 99.1232, O157:H43	(3.52 ± 0.26) B B-14573, 25253, B-2783, 35695, B-3704, B-766, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512	(3.73 ± 0.30) B B-14573, 25253, 25922, B-2783, 96.0509, 97.0147, 97.0152,[c] 97.0190, 99.0512, 99.1232	(3.07 ± 0.42) B B-14573,[c] 25253, 25922, B-2783, B-3054, 35695, B-3704, B-766, 97.0147, 97.0152,[c] 97.0190, 99.0512, 99.1232
Salmonella Montevideo G4639	(4.89 ± 0.28) A B-14573, 25253, B-2783, B-3704, B-766, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512, O157:H43	(3.60 ± 0.54) B B-14573, 25253, B-2783, 35695, B-3704, B-766, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512	(3.80 ± 0.60) B B-14573, 25253, 25922, B-2783, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512, 99.1232	(3.28 ± 0.79) B B-14573, 25922, B-766, 97.0147, 97.0152, 97.0190, 99.0512, 99.1232
Salmonella Poona RM 2350	(4.87 ± 0.31) A B-14573, 25253, B-2783, B-3704, B-766, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512, O157:H43	(3.71 ± 0.65) B B-2783, 35695, B-3704, B-766, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512	(3.38 ± 0.75) BC B-14573,[c] 25253, 25922, B-2783, 35695, B-3704, B-766, 96.0509, 97.0147, 97.0152,[c] 97.0190,[c] 99.0512,[c] 99.1232	(2.66 ± 0.84) C 11775, B-14573, 25253, 25922, B-2783, B-3054, 35695, B-3704, B-766, 96.0509, 97.0147, 97.0152,[c] 97.0190,[c] 99.0512,[c] 99.1232

[a] All *E. coli* strains, identified by strain numbers (see Table 1). Values in parentheses are the mean ± standard deviation (log CFU per gram) at each sampling point. Means within same row with the same letter are not significantly different ($P > 0.05$).

[b] *E. coli* NRRL B-2783 contains the O157 gene, and O157:H43 strains were not considered potential surrogates. These strains are included for comparison purposes only.

[c] Strains that had significantly greater ($P < 0.05$) attachment and survival than did the pathogenic strain.

TABLE 5. *Surrogate strains that demonstrate heat resistance ($D_{60°C}$) equivalent to or greater than that of the pathogenic strains*[a]

Pathogenic strain	Growth medium				
	BHI	TSB	TSB+G	TSB−G	TSB−GHCl
E. coli O157:H7 SEA 13B88	(65.99 ± 9.20)	(72.10 ± 2.62)	(90.07 ± 4.01)	(57.94 ± 12.40)	(58.72 ± 5.90)
	B-14573,[b] 25922, B-2783,[b,c] B-3054, 35695,[b] B-3704, B-766, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512, 99.1232[b] 157:H43[c]	B-14573, B-2783,[b] B-3054, 35695,[b] B-3704, B-766, 96.0509, 97.0147,[b] 97.0152, 97.0190, 99.0512, 99.1232, O157:H43	35695, 96.0509, 97.0152, 97.0190	B-14573, B-2783, B-3054, 35695, B-3704, B-766, 96.0509, 97.0147,[b] 97.0152, 97.0190, 99.0512, 99.1232, O157:43	B-14573, B-2783, B-3054, 35695, B-766, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512, 99.1232
E. coli O157:H7 OK	(72.89 ± 11.62)	(75.21 ± 13.67)	(67.82 ± 13.24)	(73.63 ± 9.97)	(68.22 ± 8.91)
	B-14573, B-2783,[b] B-3054, 35695,[b] B-766, 97.0147, 97.0152, 99.0512, 99.1232[b]	B-14573, B-2783,[b] B-3054, 35695,[b] B-766, 96.0509, 97.0147,[b] 97.0152, 97.0190, 99.0512, 99.1232	B-14573, 25922, B-2783, B-3054, 35695,[b] B-3704, B-766, 96.0509, 970147, 97.0152,[b] 97.0190, 99.0512, 99.1232	B-2783, 35695, 96.0509, 97.0147, 97.0152, 99.0512, 99.1232	B-14573, B-2783, B-3054, 35695, B-766, 96.0509, 97.0147, 97.0152, 97.0190, 99.0512, 99.132
Salmonella Montevideo G4639	(35.14 ± 6.48)	(46.50 ± 6.64)	(38.19 ± 3.77)	(47.11 ± 73)	(40.83 ± 3.50)
	11775, B-14573,[b] 25253, 25922, B-2783,[b] B-3054, 35695,[b] B-3704, B-766,[b] 96.0509, 97.0147,[b] 97.0152,[b] 97.0190, 99.0512, 99.1232,[b] O157:H43	11775, B-14573,[b] 25253, 25922, B-2783[b] B-3054, 35695,[b] B-3704, B-766, 96.0509,[b] 97.0147,[b] 97.0152,[b] 97.0190, 99.0512, 99.1232,[b] O157:H43	11775, B-14573, 25253, 25922, B-2783, B-3054, 35695,[b] B-3704,[b] B-766,[b] 96.0509,[b] 97.0147, 97.0152, 97.0190,[b] 99.0512, 99.1232, O157:H43	B-14573, 25922, B-2783,[b] B-3054, 35695,[b] B-3704, B-766, 96.0509, 97.0147,[b] 97.0152,[b] 97.0190, 99.0512,[b] 99.1232,[b] O157:H43	11775, B-14573, 25253, 25922, B-2783[b] B-3054, 35695, B-3704, B-766,[b] 96.0509, 97.0147,[b] 97.0152,[b] 97.0190, 99.0512,[b] 99.1232, O157:H43
Salmonella Poona RM 2350	(23.37 ± 2.12)	(24.51 ± 3.24)	(26.70 ± 2.91)	(23.54 ± 6.12)	(24.01 ± 1.99)
	11775, B-14573,[b] 25253, 25922,[b] B-2783,[b] B-3054,[b] 35695,[b] B-3704,[b] B-766,[b] 96.0509,[b] 97.0147,[b] 97.0152,[b] 97.0190,[b] 99.0512,[b] 99.1232,[b] O157:H43[b]	11775, B-14573,[b] 25253, 25922,[b] B-2783,[b] B-3054,[b] 35695,[b] B-3704,[b] B-766,[b] 96.0509,[b] 97.0147,[b] 97.0152,[b] 97.0190,[b] 99.0512,[b] 99.1232,[b] O157:H43[b]	11775, B-14573,[b] 25253, 25922,[b] B-2783[b] B-3054, 35695,[b] B-3704,[b] B-766,[b] 96.0509,[b] 97.0147,[b] 97.0152,[b] 97.0190,[b] 99.0512,[b] 99.1232,[b] O157:H43	11775, B-14537,[b] 25253, 25922, B-2783[b] B-3054,[b] 35695,[b] B-3704,[b] B-766, 96.0509,[b] 97.0147,[b] 97.0152,[b] 97.0190,[b] 99.0512,[b] 99.1232,[b] O157:H43[b]	11775, B-14573,[b] 25253, 25922, B-2783,[b] B-3054,[b] 35695,[b] B-3704, B-766,[b] 96.0509,[b] 97.0147,[b] 97.0152,[b] 97.0190,[b] 99.0512,[b] 99.1232,[b] O157:H43

[a] All *E. coli* strains, identified by strain numbers (see Table 1). Values in parentheses are the mean ± standard deviation $D_{60°C}$ (in seconds) for each pathogen in each growth medium.
[b] Strains that had significantly greater ($P < 0.05$) heat resistance than did the pathogenic strain.
[c] *E. coli* NRRL B-2783 contains the O157 gene, and O157:H43 strains were not considered potential surrogates. These strains are included for comparison purposes only.

ment and survival on apple surfaces, resistance to H_2O_2 wash treatment with and without storage at 4°C, growth on selective media, and thermal resistance, all situations where the strains were exposed to stressful challenges. Several strains had attachment and thermal resistance profiles similar to those seen with *E. coli* O157:H7 and *Salmonella* and would, therefore, be suitable surrogate organisms for these pathogens under such test conditions. The phenomenon of different strains of a particular organism having significantly different responses to the same stress has been reported before. Thermal resistance at 55 or 60°C of 17 different *E. coli* O157:H7 strains grown under same conditions varied significantly *(22).* Buchanan and Edelson *(6)* found different thermal resistance profiles for three different strains of *E. coli* O157:H7 heated at 58°C following growth in TSB+G and TSB−G. Thermal resistance values of *E. coli* O157:H7 SEA 13B88 in the present study following growth in TSB+G or TSB−G were 3.7- and 2.6-fold lower than those values previously reported by Buchanan and Edelson *(6)* for the same strain grown under similar conditions. These researchers determined thermal resistance at 58°C in BHI whereas we used 60°C in PW in this study. Although thermal resistance at 60°C would be lower than that at 58°C, BHI contains more solids than does PW, which could offer protection to the cells and thus result in an increase in thermal resistance *(2).* Sapers et al. *(19)* applied sanitizing agents, including H_2O_2, to apples inoculated with three different generic *E. coli* strains and one *Enterobacter* strain, and found significant variations in strain responses. These data further underline the necessity to be careful in the choice of nonpathogenic surrogate organisms.

There was a significant synergistic interaction between storage time and treatment (cell populations on apples decreased following storage at 4°C), reiterating the bactericidal effects of storing potentially contaminated fruit at refrigeration temperatures *(1, 15).* Also, survivors were more resistant to H_2O_2 wash treatment following storage at 4°C (Table 4), which could be due to biofilm formation *(4),* physiologic adaptation to stress, or survival of stress-resistant subpopulations of the organism. Although the H_2O_2 treatment significantly reduced bacterial numbers, the mean reduction was only in the range of 1 log CFU. Reduction in this range may have been achievable simply by rinsing the fruit in water, although this treatment was not included in the present study. Sapers et al. *(17, 18)* reported 1- to 2-log reductions with comparable H_2O_2 washing treatments and found greater reductions with H_2O_2 washes applied at higher temperatures (50 to 60°C).

Potential surrogate organisms for *E. coli* O157:H7 and *Salmonella* emerging from these studies include *E. coli* B-14573, ECRC 97.0147, ECRC 97.0152, ECRC 97.0190, and ECRC 99.0512 strains. These strains do not differ significantly from the pathogenic strains in their counts on either recovery or selective media (data not shown), and attachment counts were equivalent or higher than those obtained for the *E. coli* O157:H7 and *Salmonella* strains (Table 4). Further examination of the data reveals other examples of potential surrogates that differ from *E. coli* O157:H7 and *Salmonella* strains in only one or two areas and thus may be useful in certain specific situations.

Data presented here indicate that TSB and BHI are the most appropriate growth media. Overall, strains exhibited the shortest generation time, highest maximum population density, and shortest lag phase duration in TSB and in BHI. The highest thermal resistance overall was recorded for strains grown in TSB, although there are some interstrain differences, e.g., *E. coli* O157:H7 SEA 13B88 had significantly higher thermal resistance following growth in TSB+G than following growth in any other medium. *D*-values at 58°C for three different *E. coli* O157:H7 strains following growth in TSB+G were significantly higher than those following growth in TSB−G *(6).* Similarly, *D*-values at 60°C for *Pediococcus* sp. cells grown in TSB (containing 0.5% glucose) were significantly higher than those for cells grown in tryptone glucose yeast medium (containing 0.1% glucose) *(2, 3).* However, thermal resistance following growth in standard formulation TSB was not evaluated in these studies. Although growing the cells in TSB+G as an acid adaptation procedure may cause cell injury *(6, 14),* in a previous study *(5)* a large proportion of such acid-adapted cells (50 to 90%) were stressed during growth to stationary phase. Such stress was likely insufficient to affect growth in generally supportive environments, as can be seen from the growth data for strains in the present study. However, the production of excess acid during glucose metabolism may have decreased thermal resistance of surviving cells to heating at 60°C, particularly for nonpathogenic strains, which do not typically have the high acid resistance reported for *E. coli* O157:H7 *(6).* However, Annous et al. *(3)* reported that the decrease in pH of growth medium was merely indicative of glucose metabolism and was not correlated with the change in thermal resistance of *Pediococcus* sp. Similarly, in the current study growing cells in the absence of glucose (TSB−G) did not adversely affect growth rates under conditions where no other stresses were present, but the resultant cells were significantly less heat resistant than were cells grown in TSB (containing 0.25% glucose). The relatively low pH of the TSB−GHCl probably led to acid shock of the cells rather than the acid adaptation effect that cells grown in TSB, TSB+G, and BHI encounter *(3, 16).* Cells added to TSB−GHCl were probably injured during growth in this medium, as indicated by the significantly retarded growth rates, and thus were probably less resistant to subsequent heat treatment. These results further underline the necessity for the development of a well-balanced (universal) growth medium suitable for determinations of thermal *D*-values and other growth characteristics *(2, 3).*

Overall, higher cell counts were achieved with recovery media than with selective media as has been reported previously *(14),* although this effect was not significant for all strains. There were no differences in counts among five of the O55 strains and the *E. coli* O157:H7 strains on either recovery or selective media, underlining the relative resistance of these strains to the stresses applied in this study and the suitability of the O55 strains as surrogates for *E. coli* O157:H7.

E. coli ECRC 97.0152 was more similar to the *E. coli* O157:H7 strains than any other strain tested, although any of these O55 strains probably would be a good surrogate for *E. coli* O157:H7. Clonal relationship studies have established that the O157:H7 serotype is closely related to a group of O55 strains associated with infantile diarrhea *(23).* The O157:H7 serotype most likely arose from an O55:H7-like ancestor through genetic recombination events that added virulence genes to a nonvirulent *E. coli* genome *(23).* This scenario may explain the close correlation between the observed characteristics of the O55 strains and those of the O157:H7 strains in the present study.

Thermal resistance profiles observed in this study largely agreed with those reported in the literature *(6, 9, 22)* and were the only area in this study where a large degree of separation was observed between *E. coli* O157:H7 and *Salmonella* strains. Any one of the O55 strains mentioned could be a good surrogate organism to use in studies involving commodities, such as melons, that may be contaminated with both *Salmonella* and *E. coli* O157:H7 and that do not readily demonstrate thermal damage *(1).* Potential surrogate strains with equivalent or higher thermal resistance than pathogenic strains in all growth media tested and thus were considered potential surrogates for these pathogens based on this criterion include *E. coli* ECRC 97.0152, ECRC 97.0147, and ATCC 35695. Other potential surrogate strains that did not differ significantly from pathogenic strains and may be useful in certain specific situations are listed in Table 5. However, although there is some merit in erring on the side of caution by using a surrogate strain with higher thermal resistance than the target pathogenic strain (in this case, *Salmonella*), the use of such a strain could ultimately lead to unnecessary waste of heat energy and overprocessing of the target food product, with associated sensory damage, particularly if there is low likelihood of the presence of an organism as heat resistant as *E. coli* O157:H7. Therefore, difficulty arises when trying to recommend a good surrogate for *Salmonella* from the strains tested. Although *Salmonella* strains tested were among the lowest in terms of thermal resistance in this study, they had relatively high rates of attachment and survival, and very few of the nonpathogenic strains tested had the same resistance patterns. *E. coli* NRRL B-766, for example, was similar in terms of both attachment and heat resistance to *Salmonella* grown in TSB and TSB−G, but employment of this strain would significantly overestimate the heat resistance of *Salmonella* in the other growth media tested. Sensitivity to heat should be of great importance for selecting a surrogate strain in view of the limited efficacy of sanitizing washes and the success of surface pasteurization with hot water *(1, 4, 17)* or steam *(13, 20).* Although, surface pasteurization resulted in significant improvement in microbiological qualities of some fruits and vegetables and improved the shelf life of cantaloupes *(1),* it caused thermal injuries to apples *(17).* Potential surrogates that have heat resistance profiles equivalent to those of *Salmonella* Poona and *Salmonella* Montevidio strains include *E. coli* ATCC 11775, ATCC 25253, ATCC 25922, NRRL B-3054, and NRRL B-766. In this study, we found many other potential surrogates that did not differ significantly from these strains in individual growth media. Annous et al. *(1)* reported similar responses by *Salmonella* Poona RM 2350 or *E. coli* ATCC 25922 on cantaloupes to commercial-scale surface pasteurization. Thus, based on thermal characteristics *E. coli* ATCC 25922 should be an appropriate surrogate for use in evaluating the efficacy of surface pasteurization for reducing and/or eliminating *Salmonella* Poona RM 2350 on cantaloupes in a pilot plant environment. Research with additional strains of *E. coli* O157:H7 and *Salmonella* and possibly other nonpathogenic surrogates is recommended to identify a effective all-purpose surrogate organism for *Salmonella* and *E. coli* O157:H7.

ACKNOWLEDGMENTS

Many thanks are due to Laura Hansen for excellent technical assistance with the growth and thermal inactivation portions of this study, to Dr. Vijay Juneja for use of the submerged coil equipment, to Dr. Allan Pickard for analyzing the growth data, and to Dr. John Phillips for statistical analysis of results. We also thank Dr. Chobi Debroy (Gastroenteric Disease Center, Wiley Lab, Pennsylvania State University, University Park, Pa.), who performed the virulence gene detection tests on the nonpathogenic *E. coli* strains. We thank Dr. William Fett for critically reviewing this manuscript.

REFERENCES

1. Annous, B. A., A. Burke, and J. E. Sites. 2004. Surface pasteurization of cantaloupes surface inoculated with *Salmonella* Poona RM 2350 or *Escherichia coli* ATCC 25922. *J. Food Prot.,* 67:1876–1885.
2. Annous, B. A., and M. F. Kozempel. 1998. Influence of growth medium on thermal resistance of *Pediococcus* sp. strain NRRL B-2354 (formerly *Micrococcus freudenreichii*) in liquid foods. *J. Food Prot.* 61:578–581.
3. Annous, B. A., M. F. Kozempel, and M. J. Kurantz. 1999. Changes in membrane fatty acid composition of *Pediococcus* sp. strain NRRL B-2354 in response to growth conditions and its effect on thermal resistance. *Appl. Environ. Microbiol.* 65:2857–2862.
4. Annous, B. A., G. M. Sapers, A. M. Mattrazzo, and D.C. R. Riordan. 2001. Efficacy of washing with a commercial flat-bed brush washer, using conventional and experimental washing agents, in reducing populations of *Escherichia coli* on artificially inoculated apples. *J. Food Prot.* 64:159–163.
5. Buchanan, R. L., and S. G. Edelson. 1996. Culturing enterohemorrhagic *Escherichia coli* in the presence and absence of glucose as a simple means of evaluating the acid tolerance of stationary-phase cells. *Appl. Environ. Microbiol.* 62:4009–4013.
6. Buchanan, R. L., and S. G. Edelson. 1999. Effect of pH-dependent, stationary phase acid resistance on the thermal tolerance of *Escherichia coli* O157:H7. *Food Microbiol.* 16:447–458.
7. Cole, M. B., and M. V. Jones. 1990. A submerged coil heating apparatus for investigating thermal inactivation of microorganisms. *Lett. Appl. Microbiol.* 11:233–235.
8. Conner, D. E., and J. S. Kotrola. 1995. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. *Appl. Environ. Microbiol.* 61:382–385.
9. Doyle, M. E., and A. S. Mazzotta. 2000. Review of studies on the thermal resistance of salmonellae. *J. Food Prot.* 63:779–795.
10. Doyle, M. P., and J. L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. *Appl. Environ. Microbiol.* 48:855–856.
11. Food and Drug Administration. 2000. Kinetics of microbial inactivation for alternative food processing technologies. Available at: http://vm.cfsan.fda.gov/~comm/ift-glos.html. Accessed 30 October 2004.
12. Gorden, J., and P. L. C. Small. 1993. Acid resistance in enteric bacteria. *Infect. Immun.* 61:364–367.

13. Kozempel, M., E. R. Radewonuk, O. J. Scullen, and N. Goldberg. 2002. Application of the vacuum/steam/vacuum surface intervention process to reduce bacteria on the surface of fruits and vegetables. *Innov. Food Sci. Emerg. Technol.* 3:63–72.
14. Riordan, D. C. R., G. Duffy, J. J. Sheridan, R. C. Whiting, I. S. Blair, and D. A. McDowell. 2000. Effects of acid adaptation, product pH, and heating on survival of *Escherichia coli* O157:H7 in pepperoni. *Appl. Environ. Microbiol.* 66:1726–1729.
15. Riordan, D. C. R., G. M. Sapers, and B. A. Annous. 2000. The survival of *Escherichia coli* O157:H7 in the presence of *Penicillium expansum* and *Glomerella cingulata* in wounds on apple surfaces. *J. Food Prot.* 63:1637–1642.
16. Ryu, J.-H., Y. Deng, and L. R. Beuchat. 1999. Behavior of acid-adapted and unadapted *Escherichia coli* O157:H7 when exposed to reduced pH achieved with various organic acids. *J. Food Prot.* 62: 451–455.
17. Sapers, G. M., R. L. Miller, B. A., Annous, and A. M. Burke. 2002. Improved antimicrobial wash treatments for decontamination of apples. *J. Food Sci.* 67:1886–1891.
18. Sapers, G. M., R. L. Miller, M. Jantschke, and A. M. Mattrazzo. 2000. Factors limiting the efficacy of hydrogen peroxide washes for decontamination of apples containing *Escherichia coli. J. Food Sci.* 65:529–532.
19. Sapers, G. M., R. L. Miller, and A. M. Mattrazzo. 1999. Effectiveness of sanitizing agents in inactivating *Escherichia coli* in Golden Delicious apples. *J. Food Sci.* 64:734–737.
20. Tottenham, D. E., and D. E. Purser. November 2000. Apparatus and method for food surface microbial intervention and pasteurization. U.S. patent 6,153,240.
21. Whiting, R. C. 1995. Microbial modeling in foods. *Crit. Rev. Food Sci. Nutr.* 35:467–494.
22. Whiting, R. C., and M. H. Golden. 2002. Variation among *Escherichia coli* O157:H7 strains relative to their growth, survival, thermal inactivation and toxin production in broth. *Int. J. Food Microbiol.* 75:127–133.
23. Whittam, T. S., M. L. Wolfe, I. K. Wachsmuth, F. Ørskov, I. Ørskov, and R. A. Wilson. 1993. Clonal relationships among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. *Infect. Immun.* 61:1619–1629.